

The effects of different hormonal treatments on the oocyte maturation in wild grey mullet (*Mugil cephalus*) collected from the Iranian coastal waters of the Oman Sea

Arya VAZIRZADEH^{1*}, Ashkan EZHDEHAKOSHPOUR²

¹Department of Desert Regions Management, College of Agriculture, Shiraz University, Shiraz, 71441-65186, Iran.

²Off-shore Fisheries Research Center, IFRO, Chabahar, Iran.

Email: aryavazirzadeh@yahoo.com

Abstract: To investigate the effects of different hormonal treatments on the oocyte maturation in wild-caught grey mullet (*Mugil cephalus*), fish were subjected to 4 different hormonal treatments as follows: (a) a control group received 0.5 ml/kg B.W. physiological saline, (b) fish injected with 20 mg/kg carp pituitary extract (CPE) as a priming dose followed by 200 µg/kg sGnRHa as resolving dose, (c) fish injected with 200 µg/kg sGnRHa as a priming dose followed by the same injection as a resolving dose and, (d) fish received 20 mg/kg CPE as a priming dose followed by injection of 200 µg/kg sGnRHa in Freund's incomplete adjuvant emulsion. All GnRHa treated groups received 20 mg/kg B.W. metoclopramide as dopamine antagonist (DA). The interval between two injections was 24 hrs. At 6, 12, 24 and 48 hours post final injection ovarian samples were taken by soft plastic catheter and the maturation of oocytes was examined according to oocyte diameter, position of germinal vesicle and coalescence of oil droplets. The results showed that all hormonal treatments were significantly effective in progression of the oocyte maturation in comparison to control group. The treatments (b) and (d) were more effective than treatment (c) and resulted in final oocyte maturation and germinal break down. It was for the first time that emulsified GnRHa (sGnRHa-FIA) was shown to be effective in final oocyte maturation and ovulation of the species. The results showed that all hormonal treatments were effective in induction of oocyte maturation in wild-caught grey mullet and could be used in commercial hatcheries.

Keywords: Wild grey mullet, Hormone, GnRHa, Oocyte maturation, Oman Sea.

Introduction

Due to climate change, high fishing pressure and degradation of spawning grounds of commercial fishes in dry regions where man is busily removing all available sea-derived food resources, the natural populations of fish have been rigorously declined and the most likely solution to the problem and providing food for growing humane population in future is aquaculture (FAO 2012).

In a region such as Iran where fresh water deprivation is a major cultural, economic and political consideration, culture of aquatics not requiring fresh water is very important mainly in salty coastal lands where are not suitable for other agricultural activities.

Due to saline tolerance and tasty flesh as well as rapid growth, the aquaculture of grey mullet has started several centuries ago in Asia and countries along the Mediterranean Sea, however, the main source of fingerlings is still from the wild (El-Gharabawy & Assem 2006; Aizen et al. 2005).

The grey mullet is a euryhaline species spawning only in salty water but can also grow in brackish and fresh waters. However mullets, in captivity, do not spawn naturally and show some degree of reproductive dysfunction. The ovary completes vitellogenesis in captive but does not proceed with final oocyte maturation (Tamaru et al. 1994). The inability of fish in captivity to complete its reproductive cycle is most likely due to a failure at

one or more sites along the hypothalamo-hypophyseal-gonadal axis (Zohar & Mylonas 2001). The assumption was that low level of LH in pituitary or its secretion is the reason for failure of oocyte maturation and ovulation in captivity. Accordingly, co-treatment of carp pituitary extract and different analogs of GnRHa were adapted to induce ovulation (Lee & Tamaru, 1988). Aizen et al (2005) also reported that removing inhibitory effect of dopamine by antidopamines such as metoclopramide (MET) resulted in oocyte maturation and ovulation of grey mullet, implying the serious inhibitory effects of dopamines in the fish.

Many parts of Iran are covered with arid and semi-arid regions, mainly alongside the Southeastern Caspian Sea and coastal areas of the North of Persian Gulf and Oman Sea, having salty lands which are not suitable for any kinds of agricultural activities except for aquaculture. Accordingly, Fisheries Organization (SHILAT) of the country has made an effort from two decades ago with importing fingerling mullet from Egypt and Hong Kong to expand the mullet aquaculture in coastal lands of the southeast Caspian Sea in order to use barren land for job creation for coastal inhabitants. Despite several attempts with partial success, the project still has many problems and has not been fulfilled. The main problem is the lack of artificial spawning and production of live larvae (Mirhashem Rostami et al. 2006).

In 2007 a population of wild gray mullet was reported from the Chabahar coast of the Oman Sea. The objective of the present research was to study the effects of different hormonal treatments on the oocyte maturation in wild-caught grey mullet to be used as brood stock for production of fingerling instead of importing from abroad.

Material and Methods

Fish collection and husbandry: Forty fish were collected by surface seine net from coastal waters of the Oman Sea (Chabahar, Iran) during autumn 2009. Fish were transferred to hatchery while keeping in oxygenated tanks. Before introduction of fish to 10

m³ tanks, they were treated with external antibiotics (Betadine 10%), with a dose of 10 ppm in 30 minutes. The bottom of the tanks was covered with a layer of sea sand. The level of water exchange was 90% twice a day using treated sea water with salinity of 34-36 ppt. The water temperature changed from 18 to 22°C. During acclimation fish were fed with commercial shrimp feed (Havoorash, Iran).

Hormonal treatments: After 30 days of acclimation, 24 healthy fish with minimum external injuries were selected according to oocyte size (see below) and were kept in condition similar to acclimation period under natural day length. Only fish having oocyte with more than 500 µm size were selected for hormonal treatment (Tamaru et al. 1988).

Selected fish were randomly allocated to 4 groups and were injected as follows: (a) a control group received 0.5 ml/kg B.W physiological saline, (b) fish injected with 20 mg/kg B.W carp pituitary extract (CPE) as priming dose followed by 200 µg/kg sGnRHa (LHRHa2, Ningbo, Sang Sheng Pharmaceutical, China) as resolving dose, (c) fish injected with 200 µg/kg sGnRHa plus as priming dose followed by same injection as resolving dose, (d) fish received 20 mg/kg CPE as priming dose followed by injection of 200 µg/kg sGnRHa in Freund's incomplete adjuvant emulsion. The GnRHa-FIA emulsion was prepared as described before (Vazirzadeh et al. 2008, 2011). Briefly, GnRHa was dissolved in physiological saline and mixed with equal volume of Freund's incomplete adjuvant by double syringe method. All GnRHa treated groups received 20 mg/kg MET as dopamine antagonist (DA). All injections were intraperitoneal in the base of the left pectoral fin. The interval between 2 injections was 24 h. The size of oocytes before injections and details of treatments are shown in Table 1. Clove oil (with purity of 90%) in a dose of 0.01 ppm was used to anaesthetize the fish during transportation, cannulation and injection processes.

Evaluation of the effects of treatments: At 6, 12, 24 and 48 h post final injections ovarian biopsies were taken by inserting a plastic catheter (with internal

Table 1. Details of hormonal treatments in wild-caught grey mullet collected from coastal water of the Oman Sea (Chabahar, Iran). Data are shown as mean±S.D.

Treatment group	Total weight (g)	Hormone treatment (per kg body weight)		Oocyte size before injection (µm)
		1 st injection	2 nd injection	
a	1435±232	0.5ml PS ¹	0.5ml Ps	532±51
b	1346±271	20mg CPE ²	200µg sGnRH _a	568±63
c	1501±198	200µg sGnRH _a	200µg sGnRH _a	516±76
d	1520±221	20mg CPE	200µg sGnRH _a - FIA ³	553±39

The number of fish in each group was 6.

The interval time between two injections was 24 hours.

1 physiological saline; 2 Carp pituitary extract; 3 Freund's incomplete adjuvant

Table 2. The effects of different hormonal treatments on the oocyte diameter, GV position and lipid droplet coalescence in wild-caught grey mullet collected from coastal water of the Oman Sea (Chabahar, Iran). Data are shown as mean±S.D. For criteria descriptions see the text.

Treatment group	oocyte maturation parameter	Time post final injection (hrs)			
		6	12	24	48
a	Oocytes diameter (µm)	512±45	532±32	489±61	564±33
	lipid droplet coalescence	NO	NO	NO	NO
	GV position	centric	centric	centric	centric
b	Oocytes diameter (µm)	583±64	643±23	723±16	741±81
	lipid droplet coalescence	NO	NO	O	O
	GV position	centric	peripheral	migrating	GVBD
c	Oocytes diameter (µm)	564±73	571±41	589±34	601±26
	lipid droplet coalescence	NO	NO	NO	O
	GV position	centric	centric	peripheral	migrating
d	Oocytes diameter (µm)	523±34	583±25	650±43	753±69
	lipid droplet coalescence	NO	NO	O	O
	GV position	centric	migrating	GVBD	ovulation

NO= not observed; O= observed

diameter of 1.2 mm) into the genital pore and applying a slight suction to obtain an ovarian tissue sample (about 15 oocytes were sampled from each fish). The maturation of oocytes was analysed according to oocyte diameter, position of germinal vesicle (GV) and coalescence of lipid droplets (Monbrison et al. 1997; Samira et al. 2008). The egg samples were clarified in clearing sera solution (ethanol: formalin: acetic acid, 6:3:1v:v) and the stages of maturity were examined based on the germinal vesicle position as follows (Levavi-Zermansky & Yaron 1986): Stage I, central GV; Stage II, migrating GV; Stage III, peripheral GV; Stage IV, GV breakdown (GVBD).

Statistical analyses: The normality of data was checked by the Shapiro-Wilk test. The effects of hormonal treatments on the oocyte maturation were analyzed using one way ANOVA after arcsine transformation of the percentage data. Statistical significance was accepted at $P \leq 0.05$. Data are reported as means \pm standard deviation (S.D.). All statistical analyses were done using the statistical software SPSS 15.

Results

The effects of treatments on the oocyte maturation are shown in Table 2. The control fish showed no oocyte progression but all hormonal treatments

induced the oocyte maturation progress by increasing the oocyte diameter, migrating GV from the center of oocyte toward the animal pole and coalescence of lipid droplets. In fish injected with CPE and sGnRHa in saline solution the size of oocytes significantly increased compared to the groups received two injections of GnRHa in saline solution. Also no GVBD occurred in the latter group. The groups receiving CPE in the first injection and GnRHa-FIA in the second injection showed significantly higher levels of oocyte progression compared to all other hormonal treatment groups ($P \leq 0.05$). The ovulation occurred only in fish receiving sGnRHa-FIA. The lipid droplet coalescence criterion differed significantly between treatments. As for oocyte size and GV position, group b and d showed higher degrees of lipid droplet coalescence.

Discussion and Conclusions

The grey mullet is a highly valuable commercial fish which historically has problems in adaptation and spawning in captive conditions. Despite many previous attempts to induce spawning and larvae production worldwide, the aquaculture of grey mullet depends on wild collected fry (Tamaru et al. 1994; Aizen et al. 2005; Samira et al. 2008). To succeed, the aquaculture of this species needs a plan for a consistent supply of fingerlings and thus it is recommended to study the possibility of induction of spawning of wild native population of grey mullet as a reliable source of brood stocks with no requirement to importing fish from abroad.

Methods of catching, acclimation and hormonal treatment procedures developed in this study could be used as a fundamental method for further adaptation and hormonal induction of spawning in wild-caught grey mullet. Earlier studies on the reproduction of introduced grey mullet in northern Iran (Gomishan site) showed that we still have severe problems in induction of spawning and larval rearing (Mirhashem Rostami et al. 2006) and we are far away from the goal of adapting the fish in Iran.

Therefore, depending on native species instead of introduced fish can reduce the cost and shorten the way to adaptation.

In this study, results on the induction of spawning in a local population of the grey mullet were presented. In the wild, grey mullets are spawned at different periods of the year, depending on their geographical location (Abraham et al. 1966; Tamaru et al. 1989). In captivity, grey mullets do not spawn spontaneously. However, successful in and out-season spawning induction was reported in some places including in Hawaii, Taiwan and the Middle East by manipulation of temperature, photoperiod and hormone administration (Kuo et al. 1974; Tamaru et al. 1988; Aizen et al., 2005; El-Gharabawy & Assem 2006). In agreement with our observations, Monbrison et al. (2003) indicated that females with oocytes reached the diameter of 500 μm were successfully spawned after hormonal treatment. Similar results were reported by Lee et al. (1987) and Tamaru et al. (1989).

In the present study, two-step injections were for effective in induction of oocyte maturation and ovulation in grey mullet as commonly used in various fish species (reviewed by Zohar & Mylonas 2001). Commonly mullet spawned 24 h after hormonal induction but the latency period to induce GVBD and ovulation in this study was 24-48 hrs. These differences could be attributed to the type and dose of hormonal treatment, environmental conditions or differences in physical response of the local populations. It was already showed that all these factors have critical effects on the reproduction performance and latency period of induced fish (reviewed by Zohar & Mylonas 2001).

The results of this study showed that priming injection of CPE is required to induce oocyte maturation and GnRHa alone is not effective in oocyte maturation. From the beginning of the artificial spawning of grey mullet, it is reported that prime injection of GtHs (CPE or HCG) is required for induction of spawning (Tamaru et al. 1989; Aizen et al. 2005; Samira et al. 2008; Yusef et al. 2010).

GnRHa-FIA was more effective than other hormonal treatments in induction of oocyte maturation. Ovulation was also observed only in fish receiving sGnRHa as resolving dose. This is the first report worldwide showing the high potential of sGnRHa-FIA in induction of ovulation in grey mullet. Vazirzadeh et al. (2008, 2011) also reported the high potency of sGnRHa when applied in sustained delivery systems in synchronization and induction of ovulation in rainbow trout (*Oncorhynchus mykiss*) and wild caught-carp (*Cyprinus carpio carpio*). Keeping wild fish in captive condition leads to high stress in brood stocks, suppressing the gonadal maturation which needs to use higher doses of hormones than those used in domestic species (Zohar & Mylonas 2001). Research showed that, in this condition using sustained delivery methods of GnRHa is more potent than acute liquid injections of GnRHa due to prolonged constant releasing of GnRHa and repairing of HPG axis in fish (Mylonas & Zohar 2001; Vazirzadeh et al. 2008, 2009). In conclusion, the results of this study showed that combined injection of CPE as priming dose and sGnRHa-FIA plus MET as resolving dose lead to induction of oocyte maturation and ovulation of wild-caught grey mullet collected from Chabahar coastal waters of the Oman Sea. Also the results revealed that using CPE as priming dose is required for induction of ovulation comparing to two-step injection of GnRHa.

Acknowledgment

The authors would like to thank the staff of Aquaculture Department of Off-shore Fisheries Research Center due to their kind assistance during the research. We are also grateful to local Fishermen to collect wild fish.

References

- Abraham, M.; Blanc, N. & Yashouv, A. 1966. Oogenesis in five species of grey mullets (Teleostei, Mugilidae) from natural and landlocked habitats. *Journal of Zoology* 15(3-4): 155-172.
- Aizen, J.; Meiri, I.; Tzchori, I.; Levavi-Sivan, B. & Rosenfeld, H. 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *General and comparative endocrinology* 142(1): 212-221.
- El-Gharabawy, M.M. & Assem, S.S. 2006. Spawning induction in the Mediterranean grey mullet (*Mugil cephalus*) and larval developmental stages. *African Journal of Biotechnology* 5(19): 1836-1845.
- FAO 2012. The State of World Fisheries and Aquaculture. (SOFIA). Rome: Food and Agriculture Organization of the United Nations 1-230.
- Kuo, C.M.; Shehadeh, Z.H. & Milken, K.K. 1973. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, (*Mugil cephalus*) L. *Journal of Fish Biology* 5(4): 459-470.
- Lee, C.S. & Tamaru, C.S. 1988. Advances and future prospects of controlled maturation and spawning of grey mullet (*Mugil cephalus*) in captivity. *Aquaculture* 74(1): 63-73.
- Lee, C.S.; Tamaru, C.S. & Kelley, C.D. 1988. The cost and effectiveness of CPH, HCG and LHRH-a on the induced spawning of grey mullet (*Mugil cephalus*). *Aquaculture* 73(1): 341-347.
- Lee, C.S.; Tamaru, C.S.; Miyamoto, G. T. & Kelley, C.D. 1987. Induced spawning of grey mullet (*Mugil cephalus*) by LHRH-a. *Aquaculture* 62(3): 327-336.
- Levavi-Zermonsky B. & Yaron, Z. 1986. Changes in gonadotropin and ovarian steroids associated with oocytes maturation during spawning induction in the carp. *General and Comparative Endocrinology* 62: 89-98.
- Mirhashem Rostami, S.A.; Amini, K.; Joorjani, M.; Ghezel, H.C. & Shafiee, A. 2006. An investigation on artificial reproduction of *Mugil cephalus*. *Iranian Scientific Fisheries*

- Journal 14 (4): 181-196. (In Farsi, Abstract in English).
- Monbrison, D.D.; Tzchori, I.; Holland, M.C.; Zohar, Y.; Yaron, Z. & Elizur, A. 1997. Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, (*Mugil cephalus*): Preliminary studies. *Bamidjeh* 49(4): 214-221.
- Mylonas, C.C. & Zohar, Y. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Reviews in Fish Biology and Fisheries* 10(4): 463-491.
- Samira, S.A.; Alaa, A. & Mona, M.M. 2008. Reproductive biology (histological & ultrastructure) and biochemical studies in ovaries of (*Mugil cephalus*) from Mediterranean water. *Journal of Arabian Aquaculture Society* 3(1): 1- 26.
- Tamaru, C.S.; Kelley, C.D.; Lee, C.S.; Aida, K., & Hanyu, I. 1989. Effects of chronic LHRH-a + 17-methyltestosterone or LHRHa + testosterone therapy on oocyte growth in the striped mullet *Mugil cephalus*). *General and comparative endocrinology* 76(1): 114-127.
- Tamaru, C.S.; Lee, C.S.; Kelley, C.D.; Miyamoto, G. & Moriwake, A. 1994. Oocyte growth in the striped mullet *Mugil cephalus* L. maturing at different salinities. *Journal of the World Aquaculture Society* 25(1): 109-115.
- Vazirzadeh, A.; Hajimoradloo, A.; Esmaeili, H. R.; & Akhlaghi, M. 2008. Effects of emulsified versus saline administration of GnRHa on induction of ovulation in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 280(1): 267-269.
- Vazirzadeh, A.; Mojazi Amiri, B.; Yelghi, S.; Hajimoradloo, A.; Nematollahi, M.A. & Mylonas, C.C. 2011. Comparison of the effects of different methods of mammalian and salmon GnRHa administration on spawning performance in wild-caught female carp (*Cyprinus carpio carpio*) from the Caspian Sea. *Aquaculture* 320(1): 123-128.
- Yousif, O.M.; Fatah, A.A.; Krishna, K.; Minh, D.V. & Hung, B.V. 2010. Induced spawning and larviculture of grey mullet, *Mugil cephalus* (Linnaeus 1758) in the Emirate of Abu Dhabi. *Aquaculture Asia* 15(1): 1-3.
- Zohar, Y. & Mylonas, C.C. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture* 197(1): 99-136.